STUDIES OF STRAINS OF *FUSARIUM LINI*

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[Read 13 May 1948]

Contents

Introduction

It is only recently that much detailed work has been reported on the variation and variability of the flax wilt organism, *Fusarium lini*. The literature regarding the existence of strains of *F. lini* has been summarized in papers by Millikan (1945) and Borlaug (1945).

Further studies have been made in Victoria on strains of *F. lini* isolated from infected plants obtained from the D1 and D2 'wilty' areas at Drouin (Millikan, 1945). Four strains were isolated from each area. At the same time, seven of the numbered Minnesota strains of *F. lini* (Borlaug, 1945) were studied. These latter were kindly supplied by Dr. J. J. Christensen of the University of Minnesota.

Physiological Studies

Borlaug (1945) has recognized numerous cultural strains of *F. lini* distinguished by their growth on three media, namely, malt, corn meal, and potato dextrose agar respectively. Recent work by Miller (1945, 1946) has further shown that considerable variation in species of *Fusaria*, including *F. lini*, may occur due to a process termed 'cultural degradation,' whereby the naturally occurring or 'wild type' of the fungus is displaced by a mutant. The process is considered to be a degradation, as the mutants tend to be less pathogenic than the parents. The mutants also appear to crowd out the parent types, since they are more aggressive in culture.

Previously, the physiology of *F. lini* had received the attention of a number of workers, although the possibility of variation due to the existence of cultural and physiological strains of the fungus was largely unrecognized.

It is clear that *F. lini* can utilize as its sole source of carbon a variety of carbohydrates, including starch, inulin, sucrose, glucose, maltose, levulose, galactose, mannose, xylose, arabinose, rhamnose (Tochinai (1925), Anderson (1925), Reynolds (1926), White and Willaman (1928a, 1928b) and Nord and Engel (1938)). However, there is some divergence of opinion between various workers as to the relative value of these compounds as carbon sources for *F. lini*, which may perhaps be due to the fact that they were evidently working with different strains of the fungus. Nord and Engel (1938) reported that lactose was fermented much slower than maltose, while the fermentation of the latter was slower and much more uncertain than that of glucose. They detected the presence of a maltase but no lactase. The presence of a glucose oxidase was indicated by the fact that d-gluconic acid appeared in solutions of glucose or maltose. On the other hand, Tochinai (1925) claimed that maltose was the most suitable carbohydrate. Primary (Anderson (1925), Nord and Engel (1938)), and secondary (Goepfert (1941)) alcohols can also serve as carbon sources for *F. lini*.

As regards nitrogen sources, Tochinai found that nitrogen was more readily assimilated as ammonium than as nitrate, whilst nitrites were unsuitable. Reynolds (1926) on the other hand listed nitrogen sources on the basis of total nitrogen content in the following descending order of suitability for *F. lini*: KNO_3, $Ca(NO_3)_2$, ammonium lactate, dibasic ammonium phosphate, asparagin, KNO_2, $(NH_4)_2SO_4$, urea and $Ca(NO_2)_2$.

The principal metabolic products of the growth of *F. lini* in nutrient solutions are mycelium, CO_2, and ethyl alcohol. There is therefore a similarity in the enzymatic degradation of carbohydrates by living yeasts and *F. lini* (Anderson (1925), Letcher and Willaman (1926), White and Willaman (1928a, 1928b), Luz (1934), Nord and Engel (1938) and Wirth and Nord (1941)). It has been shown that pyruvic acid accumulates transitorily in the substrate, while Luz (1934) found traces of both volatile and non-volatile organic acids, including oxalic acid and tartaric acids. Letcher and Willaman (1926) studied the alcohol production of eight strains of *F. lini*, which had been found by Broadfoot and Stakman (1926) to be distinguished by their parasitic action on four varieties of flax. A correlation was indicated between alcohol production and virulence, as Letcher and Willaman found that the two forms which were least virulent produced least alcohol.

However, the degradation of carbohydrates which stops at ethyl alcohol and CO_2 with living yeasts, continues further with *F. lini* with the subsequent dehydrogenation of the alcohol produced. Goepfert (1941) has shown that ketones rather than aldehydes accumulate in the substrate.

Luz (1934) has studied the growth of *F. lycopersici* and *F. lini* in nutrient solutions, and found that the rate of growth of these fungi, as determined by the total dry weight (size) of the fungal mat, showed characteristic growth phases. The phase of logarithmic growth was followed by a phase of accelerated death rate characterised by a loss in dry weight. The phase of logarithmic growth was associated first with the formation of organic acids and active alcoholic fermentation, and

later with the gradual consumption of these products following the exhaustion of the sugar supply, while the phase of accelerated death rate coincided with the disappearance of ethyl alcohol as the main source of carbon and the appearance of ammonia, indicating that the accelerated death phase was autocatalytic in nature. This autocatalytic phenomenon has been observed to occur in other fungi by a number of workers, including Klotz (1923) in *Diplodia natalensis, Sphaeropsis malorum* and *Aspergillus niger,* Ezekiel *et al.* (1934) in *Phymatotrichum omnivorum,* and White (1943) in *Ophiobolus graminis.*

After growth for six weeks in nutrient solution, Grossmann (1934) reported that the filtrate of a culture of *F. lini* was toxic to flax at a dilution of 1:5. The toxin was extractable with methyl-alcohol from the vacuum distillation residue of the culture liquid, and was neither volatile nor thermo-labile.

Luz (1934) found that the most active substances in the causation of tomato wilt by *F. lycopersici* grown in culture solution occurred during the phase of accelerated death rate, and were therefore not direct products of sugar metabolism. Ammonia was shown to be very toxic to tomato plants.

The varying growth phases and the metabolic products associated therewith are reflected in the pH of substrate. Thus Luz (1934) observed that the reaction of the media containing *F. lycopersici* or *F. lini* showed a decline of the pH value from 3·9 to 3·5, followed by a rise to 7·5, then a fall to 7·2 or no change, and finally a rise to 8·5. Similar pH changes in the substrate may be induced by other fungi of the genus *Fusarium.* Thus Young and Bennett (1922) showed that the reaction of a culture solution in which *F. oxysporum* was grown continued to become acid until a hydrogen ion concentration of pH 3·6 was reached, then turned towards alkalinity, and growth continued until all the organic compounds were broken up and a reaction of pH 8·4 was recorded.

Working with *F. cromyophthoron,* Sideris (1925) found that for different nutrient solutions there was an 'isometabolic point', which he designated as 'that initial hydrogen-ion concentration of the culture solution of a nutrient substance which may or may not be changed slightly during the growth of the organism by the reaction of its metabolic products. The initial pH values of the cultures (of the same nutrient substance) whose position on the scale of pH values lies on either side of the isometabolic point, is changed by the reaction of the metabolic products, towards that of the isometabolic point.' With the complete consumption of the nutrient substance by the fungus, the pH of the substrate suddenly rises.

Anderson (1925) showed that *F. lini* could grow well over a wide range of initial pH values for the media of from 1·84 to 12·04, although the optimum range occurred between pH 5 and pH 7.

The above work on the pH changes in synthetic substrates induced by *Fusaria* supports the finding of Tochinai (1925) that the growth of *F. lini* in the flax plant ultimately made the sap more or less alkaline.

The vitamin requirements of *F. lini* do not appear to have received much attention. It is recognized that various species of fungi exhibit

different capacities for synthesizing vitamins or alternatively utilizing those supplied in nutrient solutions. Thus Vinson *et al.* (1945) who conducted comparative tests on the nutritive value for mice of *F. graminearum* and *F. lini* grown in culture solution, found that *F. graminearum* contained only 5 micrograms of vitamin B_1 (thiamin) per gram of dried material, and was totally inadequate for growth, whereas *F. lini* contained about 20 micrograms of thiamin per gram of dried material and proved an excellent source of this vitamin for mice for the first month. In this experiment, therefore, the capacity of *F. lini* to synthesize thiamin was greater than that of *F. graminearum*. Previously Wirth and Nord (1941) found that in nutrient solutions in which the fermentation of sugars by *F. lini* was proceeding, pyruvic acid accumulated transitorily in the complete absence of any interceptor, but that this accumulation was much less marked when vitamin B_1 was added to the solution.

Strains of the same species of fungus may exhibit differences in their capacity to utilize vitamins. Robbins (1941) and Robbins and Ma (1941) found a strain of *F. avenaceum* which failed to grow in a mineral sugar solution which constituted a satisfactory medium for two other isolates of the same fungus. However, with the addition of biotin to the medium, the first-named strain made very satisfactory growth.

The possible importance of amino nitrogen and growth factors in relation to flax wilt is indicated by the results of West and Lochhead (1940), who demonstrated that the roots of even young flax seedlings in the soil encourage the development of those types of the soil flora which are dependent on a supply of thiamin, biotin and amino nitrogen for growth, suggesting that the flax roots may secrete these substances in significant amounts. Lochhead *et al.* (1940) further showed that the rhizosphere of flax varieties susceptible to *F. lini* harboured much larger numbers of bacteria and fungi responsive to amino nitrogen and vitamins (including biotin and thiamin) than did the rhizosphere of wilt-resistant varieties.

In Victoria, the growth in nutrient solutions of the strains of *F. lini* from Drouin and Minnesota respectively referred to above, has been studied, with the object of determining whether differences between strains occurred.

A number of preliminary experiments were conducted to develop a satisfactory nutrient solution for the growth of *F. lini*. For most of these *F. lini* D1-1 was used.

General Method. The initial experiments were concerned with the effects of sugars, vitamins and amino nitrogen, and for these the composition of the base medium used was as follows:

$MgSO_4.7H_2O$	0·5 grams.
K_2HPO_4	1·0 „
$NaNO_3$	2·0 „
Dextrose	30·0 „
Iron (as $FeSO_4.7H_2O$)	1 part per million.
Zinc (as $ZnSO_4.7H_2O$)	1 „ „ „
Manganese (as $MnSO_4.4H_2O$)	0·5 „ „ „
Copper (as $CuSO_4.5H_2O$)	0·1 „ „ „
Distilled water	1,000 ml.

In some experiments KH_2PO_4 was substituted for K_2HPO_4, and in others the effects of varying the concentrations of sodium nitrate and glucose were studied.

To this solution was added vitamins and amino nitrogen as required. In making up the solution, care was taken to ensure that the final volume of solution per flask after the addition of 5 ml. of inoculum in sterile distilled water was 50 ml. and that its composition was as above. The inoculum consisted of a suspension of spores and fine mycelial fragments in sterile distilled water. The inoculum was prepared from cultures grown on potato dextrose agar or in nutrient solutions for six to ten days, and was distributed to the flasks in a transfer cabinet by means of a sterile pipette after the solutions had been sterilized by autoclaving at 5 lbs. pressure for 10 minutes. In one experiment, cultures of different ages were compared. Pyrex Erlenmeyer flasks of 200 ml. capacity were used, and there were three flasks for each treatment.

TABLE 1. — EFFECT OF INCREASING CONCENTRATIONS OF SODIUM NITRATE AND GLUCOSE ON THE DRY WEIGHT IN MILLIGRAMS OF FUSARIUM LINI D1-1 AND FINAL pH OF THE SUBSTRATE AFTER INCUBATION AT 26° C. FOR 10 DAYS.

		Glucose—Grams per Litre					Mean of 15 Flasks
		nil	10	30	60	100	
Sodium Nitrate grams per litre	0·1	**4** 7·4	**7** 4·7	**12** 4·4	**14** 4·2	**12** 4·2	**10**
	0·5	**3** 7·5	**59** 6·2	**57** 4·2	**56** 4·5	**52** 3·5	**45**
	1·0	**3** 7·6	**101** 7·5	**101** 4·3	**107** 4·6	**84** 3·5	**79**
	2·0	**3** 7·6	**112** 8·3	**165** 5·4	**178** 3·8	**189** 3·9	**129**
	4·0	**4** 7·5	**112** 8·4	**205** 5·9	**217** 4·1	**215** 3·8	**151**
	8·0	**3** 7·4	**108** 8·4	**255** 6·5	**225** 4·7	**235** 4·3	**165**
Mean of 18 Flask ..		**3**	**83**	**133**	**133**	**131**	

Figures in bold type—dry weights in milligrams.
Other figures—pH values.

Dry weight differences for significance:

	1% level	*5% level*
Glucose treatment means	7.4	5.5
Sodium nitrate treatment means	8.0	6.1
Individual means	18.0	13.6

Interaction glucose x sodium nitrate—Sig. $>$ 1%.

In the initial experiments the flasks were incubated at 26° C. for a period of eight to ten days, but later sufficient flasks of each nutrient treatment were provided to enable three flask samples to be removed at frequent intervals up to 350 hours after the commencement of the experiment.

The cultures were filtered in untared filter papers, washed with hot water, and dried in an oven at 100° C. After drying had been proceeding for a short time (approx. half an hour) the fungal mats became tough, and at this stage it was found possible to pull them out quite

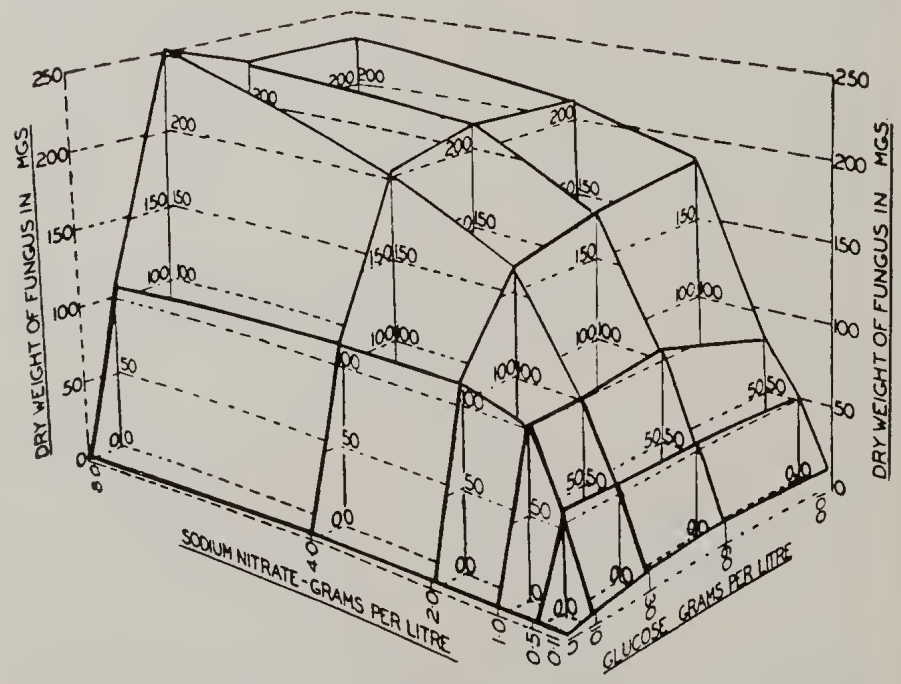

Fig. 1a.—Graph showing the effects of increasing concentrations of sodium nitrate and glucose on the dry weight of *Fusarium lini* D1-1 after incubation at 26° C. for 10 days.

cleanly from the filter papers. The mats were then rolled into balls and replaced loosely in the folded filter papers and dried to a constant weight, being weighed separately from the filter paper. The results were analysed statistically by the analysis of variance method (Fisher and Wishart, 1931).

The pH values of the original solution and of the composite filtrate from the three replicates of each treatment were determined.

Effect of Glucose and Sodium Nitrate. In this experiment the effect of simultaneous variation in the concentrations of glucose and sodium nitrate was studied. For this test, biotin concentrate was added to the solution at the rate of 1 gamma of biotin per flask. The concentrations of glucose and sodium nitrate used and the dry weights of the

fungal mats and the final pH of the substrates after incubation for 10 days are shown in Table 1 and Figures 1A and 1B.

The results show that progressive and significant increases in dry weight occurred with increasing concentrations of sodium nitrate up to 8 grams per litre. Similarly, yields increased significantly with increases in glucose concentrations up to 30 grams per litre, but no further significant increase resulted with concentrations of 60 and 100 grams per litre respectively.

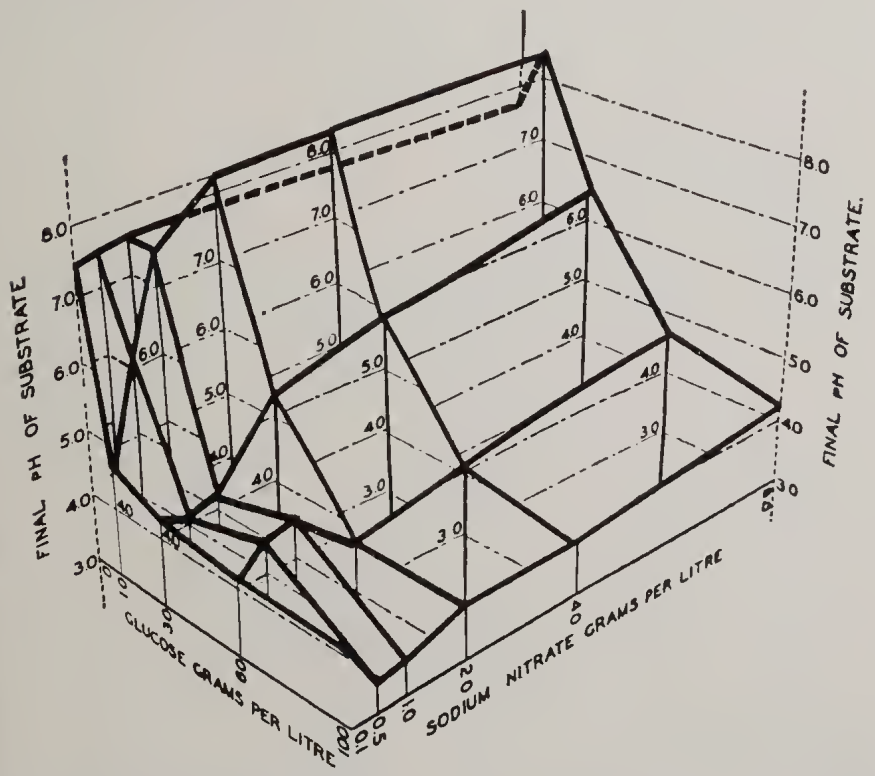

FIG. 1B.—Graph showing the effect of the growth of *F. lini* D1-1 in solutions with varying concentrations of glucose and sodium nitrate on the final pH of the substrate after incubation at 26° C. for 10 days.

However, it must be emphasized that these relative results may have varied considerably with an increase in the age of the cultures over 10 days. This is indicated by a comparison of the final pH concentrations of the various substrates shown in Fig. 1B with the pH changes in the substrate normally induced by the D1-1 strain of *F. lini* (see Figs. 4 and 5). The reaction of the substrate at the lower sugar concentrations but higher sodium nitrate concentrations had risen appreciably after 10 days, indicating the exhaustion of the carbon supply in these substrates and the actual or approaching onset of the phase of accelerated death rate, whereas the pH concentrations of the substrates at the higher

sugar concentrations were in the vicinity of 4·0, so that the fungus here was either still in the stage of logarithmic growth, or further rapid growth was being inhibited by the production of excessive amounts of metabolic products such as organic acids. It seems likely, therefore, that the fungus had attained its maximum dry weight at the lower sugar concentrations (up to 30 grams per litre) but may not have done so at the higher concentrations.

Effect of Biotin Concentrate and KH_2PO_4. The concentrations of biotin concentrate and KH_2PO_4 used are set out in Table 2 and Fig. 2. The biotin concentrate was the product of the S.M.A. Corporation, Ohio, U.S.A., and was used at rates equivalent to the concentrations of biotin indicated.

In this experiment the concentration of sodium nitrate in the base medium was 4·0 grams per litre.

Table 2.—Effect of Increasing Concentrations of Potassium Di-Hydrogen Phosphate and Biotin Concentrate on the Dry Weight in Milligrams of Fusarium lini D1-1 Incubated at 26° C. for 8 Days.

		Biotin (as biotin concentrate) gamma per flask					Mean of 15 Flasks
		nil	0·1	0·5	1·0	5·0	
KH_2PO_4 — grams per litre	nil	4	5	8	10	22	10
	0·1	73	91	95	96	84	88
	0·5	104	145	155	207	154	153
	1·5	110	158	158	162	144	146
	3·0	117	134	158	189	142	148
	6·0	119	169	153	167	129	147
Mean of 18 Flasks ..		88	117	121	139	112	

Differences for significance:

	1% level	5% level
Biotin (as biotin concentrate) treatment means	8.9	6.7
KH_2PO_4 treatment means	9.7	7.3
Individual means	21.7	16.3

Interaction biotin concentrate x KH_2PO_4—Sig. $>$ 1%.

Progressive and significant increases in yield resulted at the 0·1 and 0·5 grams per litre levels of KH_2PO_4, but no further significant increase occurred at the higher levels.

Similarly, biotin concentrate at rates up to 1 gamma of biotin per flask gave significant increases in yield compared with that of the flasks without biotin. However, the 5 gamma of biotin per flask treatment gave a significant reduction in yield over that of 1 gamma per flask.

Effect of Vitamins and Amino Nitrogen. Several experiments were conducted to examine the effects of various vitamins on the growth of *F. lini* D1-1. The composition of the base medium was as given above, and details of the vitamin treatments and the results obtained are shown in Table 3.

In these experiments, very significant increases in dry weight were induced by the addition of biotin concentrate to the nutrient solution,

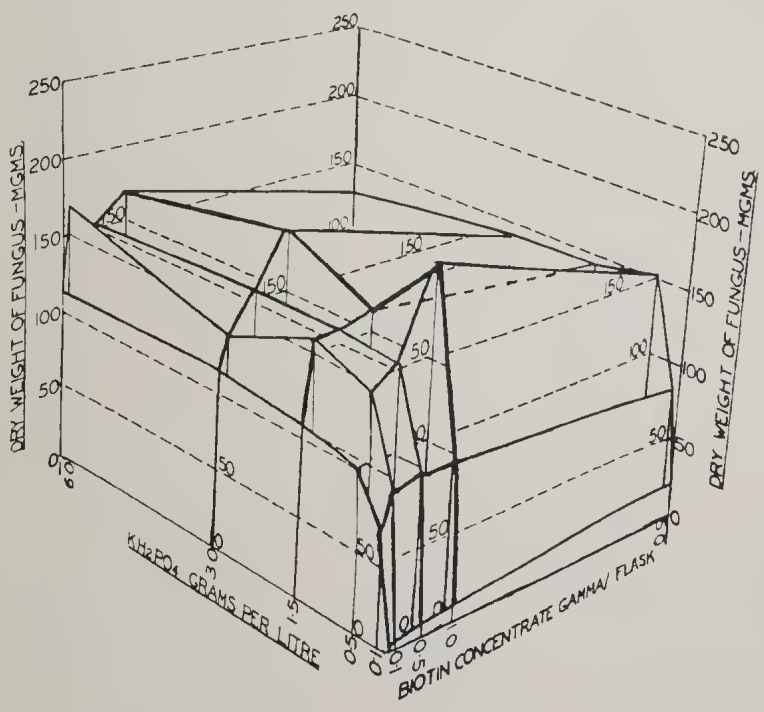

Fig. 2.—Graph showing the effects of increasing concentrations of potassium dihydrogen phosphate and biotin (as biotin concentrate) on the dry weight of *Fusarium lini* D1-1 incubated at 26° C. for 8 days.

both by itself or in conjunction with other vitamins. Thiamin, on the other hand, caused significant decreases in yield either by itself or in conjunction with biotin concentrate. Nicotinic acid, riboflavin, calcium pantothenate, vitamin B_6 hydrochloride, p-amino benzoic acid and l-ascorbic acid had no consistent significant effects on dry weight.

A further experiment was made in which the effects of biotin concentrate, crystalline biotin methyl ester and yeast were compared with and without amino nitrogen. Provision was made for three flasks of each treatment to be sampled at 65, 114 and 208 hours respectively after the commencement of the experiment. The results are shown in Table 4.

TABLE 3.—EFFECTS OF VITAMINS ON THE DRY WEIGHT OF FUSARIUM LINI D1-1 IN NUTRIENT SOLUTIONS INCUBATED AT 26° C. FOR 8 TO 10 DAYS RESPECTIVELY.

Treatment.*	Experiment No. 1		Experiment No. 2		Experiment No. 3	
	Dry Weight Mgm.	% of Base Medium	Dry Weight Mgm.	% of Base Medium	Dry Weight Mgm.	% of Base Medium
Base medium	104	100	160	100	206	100
,, ,, + Biotin concentrate ..	248	239	241	151	285	138
,, ,, + Thiamin ..	107	103	121	76	165	80
,, ,, + Nicotinic acid	114	110	209	131	218	106
,, ,, + Riboflavin ..	119	114	184	115	—	—
,, ,, + Calcium pantothenate ..	116	112	181	113	—	—
,, ,, + Vitamin B6 hydrochloride ..	118	113	164	103	—	—
,, ,, + p-amino benzoic acid ..	133	128	168	105	—	—
,, ,, + l-ascorbic acid	114	110	181	113	—	—
,, ,, + Biotin concentrate + Thiamin	185	178	199	124	—	—
,, ,, + Biotin concentrate + nicotinic acid	—	—	—	—	280	136
,, ,, + Biotin Concentrate + Thiamin + nicotinic acid ..	—	—	—	—	239	116
,, ,, + All vitamins	248	239	211	132	—	—
Differences for Significance 1% level	24	23	35	22	31	15

*Concentrations of vitamins used as follows: Biotin concentrate at rates equivalent to 1 gamma biotin per flask. All other vitamins 5 gamma per flask.

An interesting feature of these results was the significantly greater increase in yield obtained with biotin concentrate compared with crystalline biotin methyl ester. The significant increase over the base medium promoted by the latter was the same as that obtained with dried yeast. The biotin concentrate evidently contained some unknown yet highly effective growth factor.

The addition of asparagine and glycine to the base medium also caused very significant increases in dry weight.

As the result of these experiments, biotin concentrate and amino nitrogen (asparagine) were added to the base medium in all subsequent tests.

THE TIME FACTOR AND STRAINS OF F. LINI IN RELATION TO THE RESPONSE TO BIOTIN CONCENTRATE. As the above experiments with biotin concentrate had all been terminated before the completion of the

stage of logarithmic growth and the onset of the stage of accelerated death rate (particularly in the case of the control treatments), a further experiment was made in which samples were taken at intervals up to 350 hours. In this experiment also, several Drouin strains and the Minnesota strains of *F. lini* were included. The biotin concentrate was added at a rate equivalent to 1 gamma of biotin per flask. The results are presented in Fig. 3.

TABLE 4.—EFFECTS OF VITAMINS AND AMINO NITROGEN ON THE DRY WEIGHT OF FUSARIUM LINI D1-1 IN NUTRIENT SOLUTION, INCUBATED AT 26° C. FOR 65, 114 AND 208 HOURS RESPECTIVELY.

Treatment*	Dry Weight in Milligrams at :—		
	65 Hours	114 Hours	208 Hours
Base medium	1	9	28
" " + biotin concentrate	13	48	193
" " + crystalline biotin	15	26	77
" " + nicotinic acid + thiamin ..	1	8	41
" " + dried yeast	25	29	76
Base medium + amino nitrogen	1	7	86
" " + " " + biotin concentrate	13	58	317
" " + " " + crystalline biotin ..	16	27	123
" " + " " + nicotinic acid + Thiamin	2	11	73
" " + " " + dried yeast	25	45	128
Difference for significance at 1% level	2	8	28

*Details of treatments as follows:
All vitamins applied at rate of 1 gamma per flask.
Dried yeast applied at rate of 0.02 grams per flask.
Amino nitrogen consisted of asparagin + glycine each 0.2 grams/litre.

The magnitude of the response to biotin concentrate exhibited by the different strains of *F. lini* varied considerably. In the case of *F. lini* D2-4 and the Minnesota strains 4, 8, 11 and 28 very significant growth responses to biotin concentrate occurred at 150 hours, whereas no significant response occurred at this stage with D1-2, 6 and 27 strains. However, irrespective of any initial response to biotin concentrate, all strains showed no significant differences in either maximum dry weights or the final dry weights at 350 hours between the base medium and the base medium plus biotin concentrate.

It is evident, therefore, that while the growth factors present in biotin concentrate will cause an initial response in certain strains of *F. lini*, all strains are capable, in time, of supplying their own needs of these factors.

The pH changes in the substrate shown in Fig. 3 will be discussed in a later section of this paper.

Comparative Growth of Four Strains of F. lini in Dextrose and Sucrose Solutions. An experiment was made in which the efficacy of dextrose and sucrose as carbon sources for four strains of *F. lini* were compared. The following modifications to the base medium as set out above were made for this experiment: K_2HPO_4 2·0 grams, $NaNO_3$ 4·0 grams, and asparagine 0·4 grams per litre. Dextrose and sucrose were added to the respective solutions at the rate of 30 grams

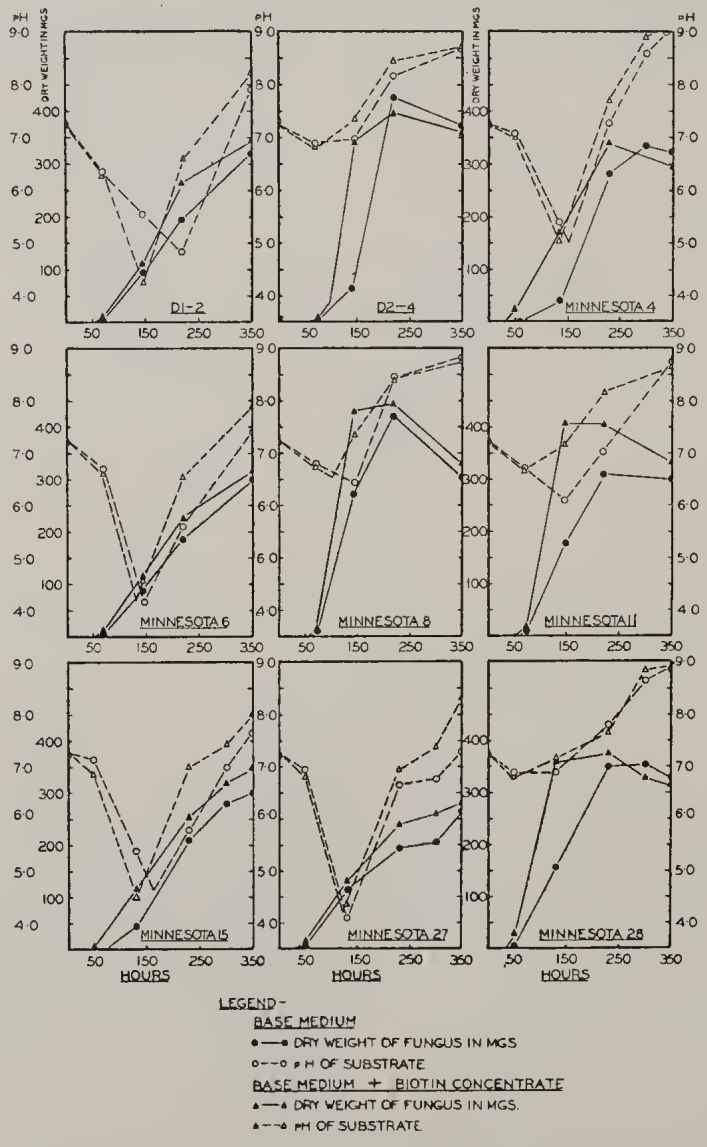

Fig. 3.—Results of experiment with nine strains of *Fusarium lini*, showing the effect of time in relation to the response to biotin concentrate.

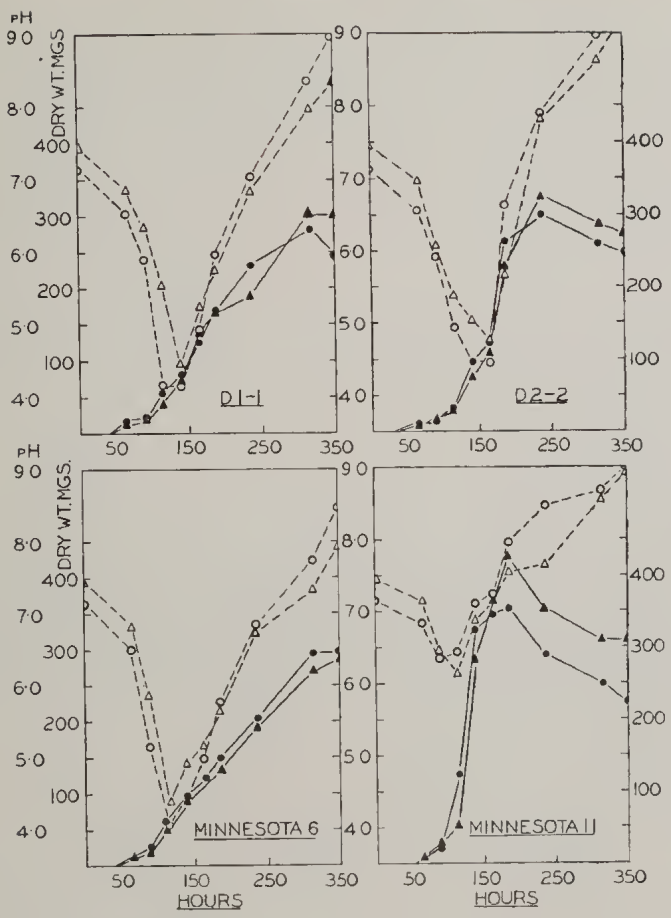

FIG. 4.—Effect of nutrient solutions containing either dextrose or sucrose on the dry weights of, and pH changes in the substrate induced by, four strains of *Fusarium lini* incubated at 26° C.

per litre. Twenty-seven flasks of each sugar treatment for each strain were provided, enabling a three-flask sample to be taken at nine successive intervals up to the termination of the experiment at 350 hours. The results of dry weight and pH determinations made on these samples are given in Fig. 4.

The results obtained with three of the strains of *F. lini* used, namely D1-1, D2-2 and Minnesota 6, were identical in each case for the dextrose

and sucrose solutions, although differences between the strains occurred. With the Minnesota 11 strain the results were similar in the two types of sugar solutions during the stage of logarithmic growth, but the sucrose medium later gave significantly higher values for the maximum dry weight at 188 hours and during the following period of accelerated death rate. The Minnesota 11 strain also differed considerably from the other three strains in the pH changes induced in the media. This phenomenon will be discussed in more detail in a later section of this paper.

Effect of the Initial pH of the Substrate on Growth. An experiment was made in which *F. lini* D1-1 was grown on a medium adjusted to various initial pH values. The composition of the solution was as follows:

Peptone (5·5% H_2O)	3·3 grams.
Beefine (56% H_2O)	7·0 „
Glucose	10·0 „
Distilled water	1,000 ml.

During the pH adjustment, the volume of the solution was increased 10 per cent. Erlenmeyer flasks of 100 ml. capacity were used, and 20 ml. of the adjusted nutrient solution was added to each, there being three flasks to each treatment. The pH of each solution was obtained after sterilization and immediately before inoculation. The inoculated flasks were incubated at 26° C. for six days, when the experiment was terminated. The initial pH treatments and the dry weights obtained are shown in Table 5.

Table 5.—Effect of the Initial pH of the Nutrient Solution on the Dry Weight of Fusarium lini D1-1, Incubated at 26° C. for 6 Days.

Initial pH	Final pH	Dry Weight Mgms.
1·96	1·88	0
2·73	2·44	20
3·00	2·49	39
3·74	3·25	41
4·05	3·64	49
4·61	4·17	52
5·06	4·40	48
5·53	4·79	53
6·20	4·88	48
6·33	5·36	53
6·60	6·13	49
7·20	6·54	49
7·60	7·05	46
Difference for significance 1%		3

These results differ slightly from those of Anderson (1925) cited above in that no growth was obtained in the above experiment at an initial pH of 1·96, whereas Anderson recorded growth at pH 1·84. A variation in the strain of *F. lini* used in these two experiments could possibly account for this difference. Apart from this the experiment confirms that *F. lini* will grow over a wide range of initial pH values.

Comparative Growth of Strains of *F. lini* in Nutrient Solutions

Modified Method. Following the preliminary experiments set out above, the composition of the base medium used for the comparative growth studies of *F. lini* strains was modified as follows:

$MgSO_4.7H_2O$	0·5 grams.
$NaNO_3$	4·0 „
Asparagine	0·4 „
Dextrose	30·0 „
Zn (as $ZnSO_4.7H_2O$)	1 part per million.
Fe (as $FeSO_4.7H_2O$)	1 „ „ „
Mn (as $MnSO_4.4H_2O$)	0·5 „ „ „
Cu (as $CuSO_4.5H_2O$)	0·1 „ „ „
Biotin (as biotin concentrate)	0·5 gamma per flask.
Distilled water	1,000 ml.

To the above base medium was added one of the following:

K_2HPO_4	2·0 grams.
KH_2PO_4	2·0 „

The pH of the base medium containing the first of these phosphate sources (termed the K_2 solution) was approximately 7·2, and that containing the second (the H_2 solution) approximately 4·5.

Each strain was grown in both the K_2 and H_2 solutions, and sufficient flasks of each were provided to enable three-flask samples to be taken every 24 hours during the greater part of the experiment. However, the first sample was not taken until approximately 60 hours after commencement of the test, as little growth occurred before this time. Other details of procedure were the same as those described in the general method above.

Results. Each strain was studied in three separate tests, and similar results were obtained in each instance. The results from one such test are presented in Fig. 5.

On comparing the results shown in Figs. 3, 4 and 5, some marked differences between the strains of *F. lini* are apparent.

The rates of growth of the strains, as indicated by the dry weights, showed considerable variation. The strains D1-1, D1-3, D1-4, D2-2, D2-3, D2-4, Minnesota 8, 11 and 28, passed through the stage of logarithmic growth rapidly, and reached the maximum dry weight in from 140 to 200 hours, according to the strain involved. Differences in the magnitude of this maximum dry weight occurred between strains. By the end of 350 hours all the above strains were well into the stage of accelerated death rate. The remaining strains, namely D1-2, D2-1, Minnesota 4, 6, 15 and 27, showed characteristically slow rates of growth and in most cases were only just entering the phase of accelerated death rate at the termination of the test.

When the relative rates of growth of the strains are considered in conjunction with the concomitant pH changes induced in the substrate, the differences between strains is more marked. A general feature of the pH changes is at once apparent. Irrespective of the different initial pH values of the K_2 and H_2 solutions, the values of the isometabolic points in the two solutions sown to any one strain are very comparable.

The rise in pH occurs simultaneously in both solutions, and the subsequent changes in pH values are usually almost identical.

On the basis of the type of pH change induced, the strains may be divided into two groups. In the first group are the strains D1-1, D1-2, D2-1, D2-2, D2-3, Minnesota 4, 6, 15 and 27, which induce pH changes similar to those described for *Fusaria* by various workers cited above, i.e., a considerable drop (particularly in the K_2 medium) to an isometa-

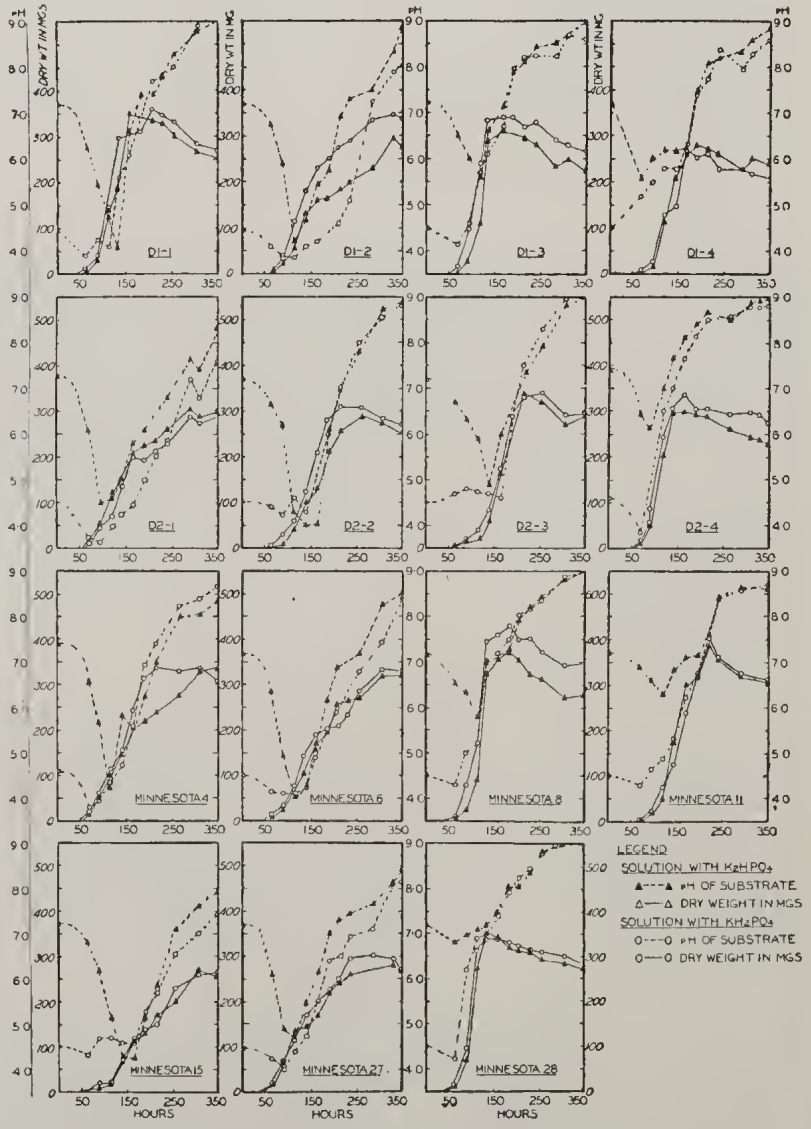

FIG. 5.—Comparative growth of 15 strains of *F. lini* in two nutrient solutions of different initial pH incubated at 26° C.

bolic point, followed by a rapid rise to a pH of 8 or more. However, differences occurred between these strains in the relative values of the isometabolic points for the K_2 and H_2 solutions. Some of the strains included in this first group with low isometabolic points, namely D1-1, D2-1, D2-2, D2-3, Minnesota 4, 6, 15 and 27, which induce pH changes while the remaining strains show the slower type of growth.

The second group of strains, namely D1-3, D1-4, D2-4, Minnesota 8, 11 and 28, are characterized by a high pH value for the isometabolic point. These strains all pass rapidly through the logarithmic growth phase.

These differences in growth rates and in the isometabolic points characteristic of the different strains of *F. lini* studied in these experiments indicate the existence of important physiological differences between them. These strain differences are evidently reflected in the nature and amounts of the metabolic products, including organic acids and ethyl alcohol, produced. Letcher and Willaman (1926) have already reported differences in the amount of alcohol produced by eight strains of *F. lini*. Unfortunately, no detailed study was possible of the amounts of alcohol and nature of the other metabolites formed by the *F. lini* strains in the present experiments. Qualitative tests indicated the presence of alcohol most strongly between 140 and 180 hours, depending on the strain involved. The attainment of a pH value of approximately 7·5 to 8·0 in the substrate coincided with the onset of the phase of accelerated death rate, and it is probable from the literature citations given above that at this stage the alcohol had disappeared completely, and that autocatalytic reactions had commenced. At 350 hours, when the pH values were in the vicinity of 9·0, ammonia was present in the substrate.

Limited toxicity tests were conducted with filtrates from cultures inoculated with the D2-2, D2-3, Minnesota 8 and 15 strains. These filtrates were obtained at 164, 188, 305 and 350 hours, and were passed through a Seitz filter before being added to the nutrient solution described by Arnon (1938) in which flax seedlings were growing. The ratios of filtrate to nutrient solution tested in each case were 1 : 3, 1 : 5 and 1 : 10 respectively. Wilt symptoms were produced by all filtrates from each time of sampling, the severity decreasing with increasing dilution. First signs of wilt occurred after three days. The filtrates from the K_2 solutions proved to be more toxic than those from the corresponding H_2 solution.

The age of the inoculum was found to affect the value of the isometabolic point and the rate of growth of any particular strain. The use of inoculum from old staled cultures, or inoculum stored in distilled water for several weeks or months, resulted in very slow growing cultures, in which the drop in pH of the substrate was much greater, and the subsequent rise much slower than that which occurred in cultures from fresh inoculum of the same strain. For this reason inoculum for comparative studies between strains was always obtained from young actively growing cultures.

Staling of the cultures may also affect their virulence to flax. Thus Pavlushin (1937) found that cultures of *F. lini* which had grown for

nearly six months on artificial media failed to produce wilt in flax when used in inoculation tests, whereas one month old cultures of the same isolate were strongly virulent.

Varietal Reaction to Strains of *Fusarium lini*

In previous studies of two forms of *F. lini* designated D1 and D2 respectively, isolated from two different crops of wilted flax in the Drouin district of Victoria, Millikan (1945) concluded from the results of tests with numerous flax varieties and selections that these strains were distinct from each other and also from two forms of *F. lini* from New Zealand, and one from Minnesota.

Similarly, as a result of an extensive investigation conducted at Minnesota, Borlaug (1945) demonstrated that the species *F. lini* is composed of a large number of races which differ in cultural characteristics, pathogenicity on flax varieties, temperature requirements, and compatibility. It was found that a variety may be completely susceptible to one race, but resistant to another race, and vice versa. An antagonistic phenomenon among races of *F. lini* was also observed. Thus the amount of wilt produced in a variety when inoculated singly with either of two such races was found to be much greater than the amount produced when the same variety was inoculated with a mixture of these two races. For this reason, some varieties which have appeared to be resistant in the wilt nursery have been proved in greenhouse tests to be susceptible to individual races isolated from the same wilt nursery.

In conjunction with the physiological studies reported above, the strains of *F. lini* were tested to confirm their pathogenicity to flax, and further to determine the reactions of certain flax varieties to them.

Method. The respective strains of *F. lini* were grown for ten days in nutrient solution. The cultures were then filtered and washed, and the appropriate mycelial mats were added to the top three inches of pots of steam-sterilized soil, maintained at a constant temperature of 75° F. in the Burnley Temperature Tanks. Three pots were inoculated with each particular strain. A sowing of the susceptible Concurrent variety (Millikan 1945) was made in all pots immediately after inoculation, to assist in increasing the wilt infection in the soil. Approximately two weeks after emergence of the Concurrent, very satisfactory wilt development had occurred, the actual amount in each pot depending on the virulence of the particular strain concerned. The remaining non-wilted Concurrent plants were then removed, and the surface of each pot was divided into four equal areas by means of short sticks.

A sowing of Concurrent was made in one of these sections in each pot for control purposes, and the remaining three sections were each sown with a different test variety.

After emergence, and before the appearance of wilt symptoms (which usually occurred in from four to eight days) the seedlings in each section were counted. Later the wilted seedlings were counted and removed at frequent intervals up to 50 days after emergence, when the experiment was terminated. The percentages of wilt based on the emergence figures

were then calculated for each count and from this data the curves shown in Figs. 6 and 7 were drawn.

Results from a few pots in which the Concurrent controls did not develop a satisfactory percentage of wilt (compared with that in the control sowings in the other pots inoculated with the same strain of *F. lini*) were discarded.

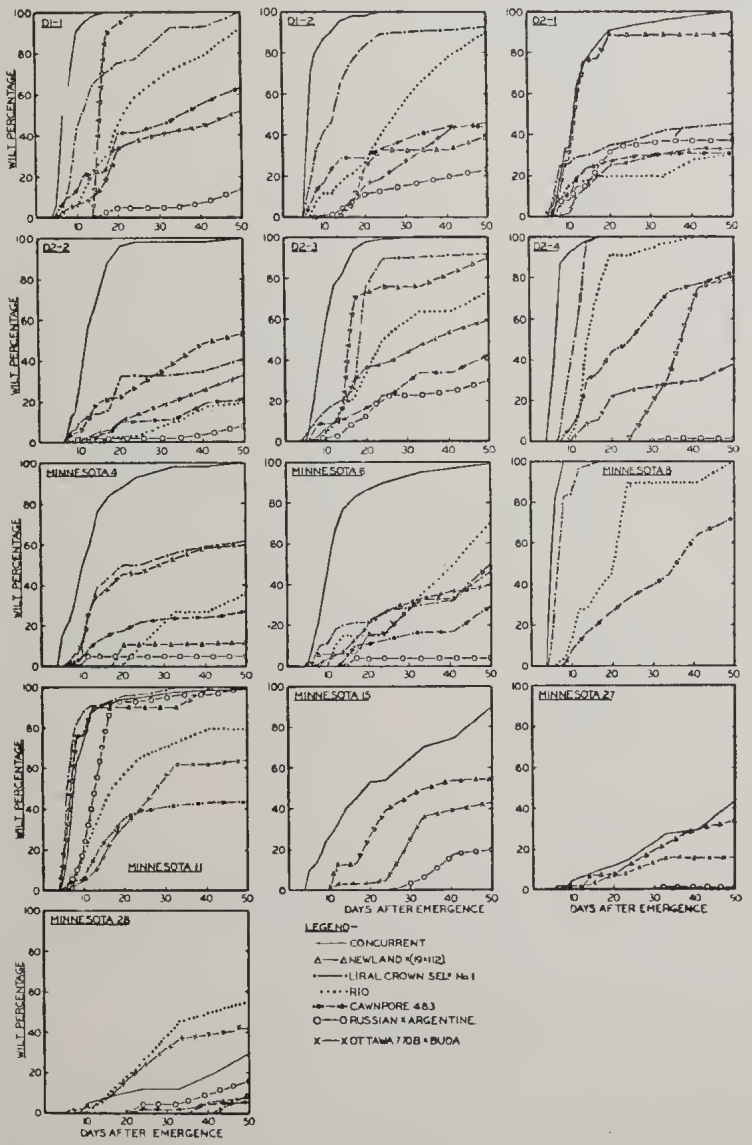

FIG. 6.—Results of Experiment No. 1, showing the relative wilt reactions of flax varieties grown in sterilized soil inoculated separately with six Victorian and seven Minnesota strains of *Fusarium lini* respectively, and maintained at a constant temperature of 75° F.

Results. Two separate experiments were made, the results of which are presented in Figs. 6 and 7 respectively. The strains were tested in soil maintained at 75° F. to provide standard conditions for comparison with the results from previous tests (Millikan, 1945) in which the same varieties were grown in naturally infected soil maintained at this temperature.

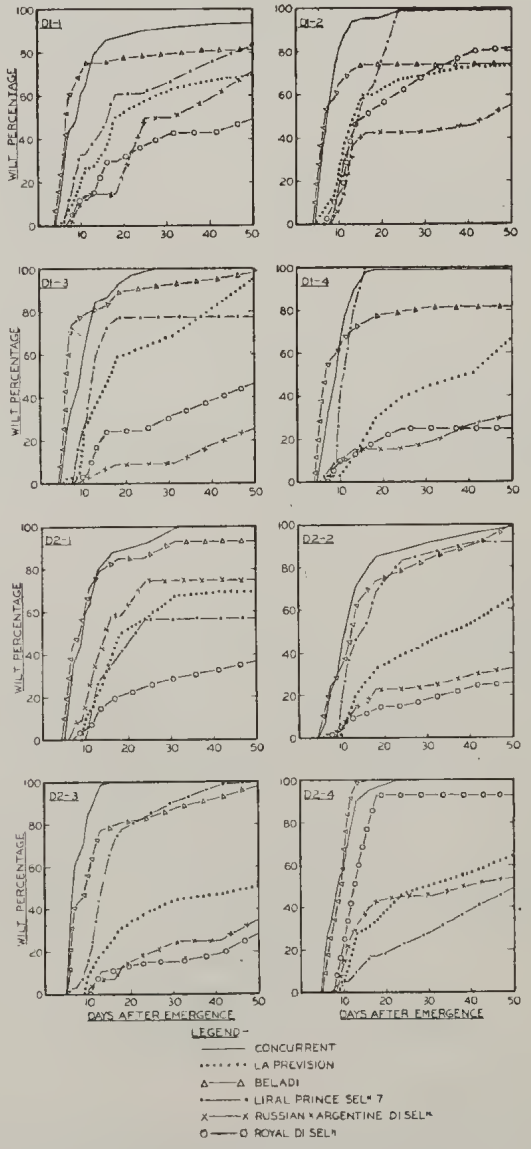

Fig. 7.—Results of Experiment No. 2, showing the relative wilt reactions of flax varieties grown in sterilized soil inoculated separately with eight Victorian strains of *Fusarium lini*, and maintained at a constant temperature of 75° F.

From the results of these two experiments, the following conclusions are drawn. All the strains of *F. lini* used in the physiological studies reported above were shown to be pathogenic to flax, although the general virulence of certain strains, e.g., Minnesota 15, 27 and 28, to the varieties studied was not as great as that of the remaining strains. However, pathogenicity could not be correlated with the values of the isometabolic points characteristic of the various strains when grown in nutrient solutions. Whereas some strains with high isometabolic points, e.g., Minnesota 8 and 11, proved to be highly pathogenic, the Minnesota 28 strain, which also showed a high isometabolic point, was much less pathogenic to the varieties tested.

Differences in the relative wilt development in the flax varieties tested occurred between strains. With the exception of the less pathogenic strains, Minnesota 15, 27 and 28, all strains concerned showed indentical reactions to the varieties Concurrent, Beladi and La Prevision, whereas the remaining varieties showed considerable differences in their relative susceptibilities to the strains.

A striking result occurred in the case of Russian x Argentine, which proved highly susceptible to Minnesota 11 and very resistant to all the other strains (with the exception of Minnesota 8, which was not included in this instance). However, similar differences were found to exist between other strains with other varieties. For example, the strains D1-1 and D1-2 produced identical wilt development in the varieties Ottawa 770B x Buda (moderately resistant), Russian x Argentine D1-Selection (moderately resistant), Rio (susceptible) and Liral Crown Selection 1 (very susceptible), whereas the variety Newland X (19 x 112) was very susceptible to D1-1 and resistant to D1-2.

From the results of the above two experiments, it is concluded, therefore, that various isolates of *F. lini* concerned could be distinguished from each other on the basis of their relative pathogenicity to flax varieties.

The above differences in the wilt reactions of the flax varieties to the Victorian D1 and D2 strains of *F. lini* are of considerable significance, and fully confirm previous conclusions by Borlaug (1945) that such strains isolated from the same wilt nursery may show large differences in their relative pathogenicity to selected flax varieties. A variety may be resistant in the nursery, yet susceptible to individual strains of *F. lini* isolated from the nursery soil.

Previous tests with naturally infected D1 and D2 soils under the same temperature conditions (Millikan, 1945) had shown that the varieties Newland X (19 x 112) and Royal D1 Selection were very wilt resistant, whereas these same varieties proved susceptible to certain individual strains of *F. lini* isolated from the D1 and D2 areas respectively.

On the other hand, other varieties, e.g. Liral Crown Selection 1 and Rio, proved more resistant to certain individual Victorian strains than to the combined wilt flora present in the same naturally infected soil from which the strains were isolated.

From the results obtained in the above and in previous experiments (Millikan, 1945) it appears evident that the duration of wilt tests should be sufficiently long to enable all susceptible individuals to manifest symp-

toms, thus enabling a proper assessment of the wilt reaction of the variety under test to be made. For this reason, the writer's temperature tank experiments were conducted for 50 days after emergence, while the duration of field experiments, particularly those sown in winter in which 'late wilt' symptoms developed, was considerably longer. In all cases it was possible to isolate *F. lini* from plants showing 'late wilt' symptoms, whether grown in the temperature tanks or in the field, and the presence of the fungus in the aerial parts of such plants was readily detected (Plate I). Results of wilt resistance tests terminated only two or three weeks after emergence may therefore not give a true indication of the relative wilt resistance of flax varieties.

Summary

Comparative studies of eight Victorian and seven Minnesota strains of *F. lini* have been made.

The effects on the growth of *F. lini* D1-1 of increasing concentrations of sodium nitrate, glucose, potassium di-hydrogen phosphate and biotin concentrate respectively in the nutrient solution were determined.

The vitamin nutrition of this strain was also studied. Significant increases in growth occurred with the addition of biotin concentrate, crystalline biotin methyl ester and dried yeast, although by far the greatest growth response followed the addition of biotin concentrate. Other members of the vitamin B complex and l-ascorbic acid gave no significant response, except thiamin, which reduced the yield.

The magnitude of the response to biotin concentrate exhibited by nine different strains of *F. lini* varied considerably. However, irrespective of any initial response, all strains showed no significant differences in either maximum dry weights or final dry weights at 350 hours between the controls and biotin concentrate treatments. All strains were evidently capable, in time, of supplying their own needs of the growth factor(s) present in biotin concentrate.

The efficacy of dextrose and sucrose as carbon sources for four strains of *F. lini* were compared. With three of the strains no significant differences occurred between the dextrose and sucrose solutions. With the fourth strain (Minnesota 11) the sucrose medium gave significantly higher values for the maximum dry weights and during the following period of accelerated death rate.

Growth of *F. lini* D1-1 occurred in solutions with initial pH values ranging from 2·73 to 7·60. No growth occurred at an initial pH of 1·96.

The comparative growth of 15 strains of *F. lini* in two nutrient solutions of different initial pH was studied. In general two stages of growth were recognized, viz., a stage of logarithmic growth to a maximum dry weight, followed by a stage of accelerated death rate characterized by a loss in dry weight. Considerable variation in the rates of growth, as indicated by the dry weights, occurred between strains.

Wide differences also occurred between strains with regard to the pH changes which their growth induced in the substrate. One group of strains was characterized by a low pH value for the isometabolic point, and the other group by a high pH value for this point. The

attainment of a pH of 7·5 to 8·0 in the substrate coincided with the onset of the phase of accelerated death rate.

The age of the inoculum appreciably affected the rate of growth and pH changes in the substrate.

Typical wilt symptoms in flax were produced by filtrates of cultures.

All strains of *F. lini* included in the physiological studies were shown to be pathogenic to flax.

Differences in relative wilt development in the flax varieties tested occurred between strains. The various isolates of *F. lini* could therefore be distinguished from each other on the basis of their relative pathogenicity to flax varieties.

A flax variety may be resistant to wilt when grown in naturally infected soil, yet susceptible to an individual strain of *F. lini* isolated from that soil, and vice versa.

In any one flax variety, considerable differences may occur between wilt-susceptible individuals growing in infected soil, in the time taken to develop wilt symptoms. The duration of wilt tests should therefore be sufficiently long to enable all susceptible individuals to develop symptoms.

Acknowledgment

This investigation was conducted in the Biological Branch of the Department of Agriculture, Victoria.

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Description of Plate

Plate I.—Transverse (upper) and longitudinal (lower) sections through stem, at 3 in. above ground level, of flax plant showing 'late wilt' symptoms. Mycelium and spores of *Fusarium lini* occur in vascular tissues.